GENOME-WIDE PROFILING OF COPY NUMBER ALTERATIONS IN CANCER: FOCUS ON MELANOMA

Luigi Pasini

Laboratory of Translational Genomics, Centre for Integrative Biology (CIBIO), University of Trento, Italy

*Thanks to a never-before detailed view of the human genome, the last decade has brought to light the notion of DNA copy number variation (CNV) as the pivotal force contributing to population genomic diversity and evolution. It is as well clear now that cancer typically results in loosened control over genomic integrity and that the acquisition of somatic copy number alterations (SCNAs), whether confined to specific genes or affecting entire chromosome arms, is likely to be a fundamental prerequisite to the adaptive pressure that drives oncogenesis. This review gives a brief overview of key developments in genome-wide SCNA profiling, with specific emphasis on array-based techniques and deep-sequencing, which indeed enabled us to identify the large majority of genomic regions undergoing frequent alteration in human cancers and defining recognizable clinical phenotype. Alongside with the prospective to take advantage for future personalized precision medicine, high-throughput SCNA analysis have already proven diagnostic and prognostic potential, particularly for those clinically unpredictable and therapy-refractory tumors, such us cutaneous melanoma. **Biomed Rev** 2013; 24: 11-24*

INTRODUCTION

Germline copy number variation (CNV) of genes and single nucleotide polymorphism (SNP) are two major driving forces of biological evolution, contributing substantially to the genomic diversity between phenotypically normal individuals and conferring susceptibility to a variety of human diseases including cancer. However, CNVs are estimated to happen more frequently (10^{-5}) compared to single-nucleotide substitutions (10^{-6}) or single nucleotide insertion/deletion (10^{-7} - 10^{-8}), giving potentially the strongest contribution for selective pressure (1). CNVs include deletions, duplications, insertions, and amplification that are in the range between 1 kb and entire chromosome arms (1-4). These type of structural variations collectively constitute the so-called copy number polymorphism (CNP), which generally account for less than 1% of the genomic variability in the human population (5-7). Possibly thousands of variable loci exist, comprising as much

Correspondence to Dr Luigi Pasini, Laboratory of Translational Genomics, Centre for Integrative Biology, University of Trento, Via delle Regole 101, 38123 - Trento, Italy. Tel.: +39 0461 283283, Fax: +39 0461 283937, Email: luigi.pasini@unitn.it

as 12% (about 360 Mb) of the entire genome (2), with a considerable fraction (>20%) involving protein coding genes with known or putative functional relevance (1,2).

Excessive changes in DNA copy number may have adverse influence on fitness and are, for the most part, highly detrimental, which keeps the population frequency of germline CNVs lower than 1% (6,7). Consequently, predisposition to diseases is possibly driven by cumulative effect of single CNVs of multiple genes, which individually would have little or no influence. However, under strong selective pressure, such as that acting during neoplastic transformation, most of the genomic rearrangements involve individual genes or set of specific genes with strong phenotypic impact (8).

Amplification of oncogenes and deletion of tumor-suppressor genes are indeed the major drivers of tumorigenesis and the massive acquisition of somatic copy number changes, called somatic copy number alterations (SCNAs), is a characteristic of all tumors (8,9). During the last decades, several methodologies have been developed to improve the resolution of genomic alteration analysis, bringing to light that there is a great variability in the number of SCNAs in human tumors (see the Mitelman Database of Chromosome Aberrations in Cancer, http://cgap.nci.nih.gov/Chromosome/Mitelman), depending on the tumor type and the degree of aggressiveness, although structural mutations that give the strongest survival benefit are highly common across cancer.

This review will make a point of how the introduction of ever-more detailed genomic profiling platforms based on array comparative hybridization (aCGH) and next-generation sequencing (NGS) have changed the understanding of the complex landscape of genome instability in cancer with important consequences for the diagnostic analysis and the prediction of oncogenic progression. Specific attention will be given to cutaneous malignant melanoma (MM), for which conventional methods and traditional cytogenetic analysis do not always allow accurate preventive diagnosis of a tumor that, if taken at the earliest stages, will have elevated chances of favorable prognosis.

BIOLOGICAL IMPLICATIONS OF SCNAS

Hotspot changes in single or small set of genes as well as large-scale SCNAs, including several megabases' segmental alterations and whole-chromosome aneuploidies, constitute to the greatest part of genomic instability observed in cancer

(9), and can have dramatic effect on the perturbation of many cellular pathways (10). Acquisition of SCNAs is potentially the most important contributor to the increased fitness of cancer cells under imposed selection and can promote rapid and specific resistance to therapeutic agents. Currently, non-allelic homologous recombination (NAHR), which involves crossing-over between highly homologous DNA segments or tandem sequences of variable numbers of tandem repeats (VNTRs), during cell division, and non-homologous end joining (NHEJ), which is a highly conserved double strand DNA repair mechanism, are the best-studied routes through which SCNAs can be generated (9,11). Deregulation of these processes is typically facilitated in a context of polyploidy and consequent aneuploidy, which may represent the initial steps of genetic adaptation promoting oncogenesis (12). During continuous selective pressure, chromosomal aneuploidy is replaced by more subtle genetic changes, mainly SCNAs that achieve the same goal with less adverse effect than whole-chromosome gain/loss (13,14). Mutations that may suppress the detrimental consequences of genomic instability may also enhance the survival advantage conferred by SCNAs. Indeed loss of p53 is a major contributor to tumorigenesis (15) by inducing toleration to the DNA-damage response that follows genomic rearrangements and chromosome mis-segregation (16,17).

As a consequence, most advanced tumors usually present much complex patterns of molecular rearrangements in their genome, generally due to breakage of chromosomes during cytokinesis and inefficient replication (18). In some cases these defects culminate in the extreme phenomenon of chromothripsis, a recently discovered and rare catastrophic event that can randomly affect one or a few chromosomes in about 2-3% of tumors (19,20). This feature is associated to most aggressive prognosis in patients and is potentially due to the formation of extra-genome micronuclei (double minutes) after chromosome segregation defects that undergo further fragmentation and rearrangements (21).

Altogether these karyotype changes contribute to the dramatic scenario observed in cancer. Whether eliciting cumulative phenotypes that are independent of the identity of the genes involved or inducing direct effects on the expression level of specific genes, duplications, amplifications, deletions, and whole-chromosome gains or losses are the hallmark of cancer and are apparently much more pivotal and frequent compared to single-nucleotide alterations, than previously thought.

FROM TRADITIONAL METHODS TO HIGH-THROUGHPUT WHOLE-GENOME MAPPING OF SCNAS

Conventional cytogenetic banding techniques usually allow the detection of segmental alterations not smaller than 5-10 Mb (22), which limits the analysis only to gross, well-recognizable chromosomal rearrangements. With the introduction in the 1990s of molecular approaches, such as fluorescence *in situ* hybridization (FISH), into the clinical testing and genetic research it was possible to discover a number of novel genomic micro alterations by the ability of hybridizing specific fluorescent DNA-probes on metaphase chromosomes or interphase nuclei (23). However, the sensitivity of FISH is limited by both the size of the probe and by the fact that only regions recognized by specific probes can be queried. Similarly to FISH, the use of traditional genomic comparative hybridization (CGH) employs metaphase chromosome as the hybridization target and is unable to reveal submicroscopic alterations that involve less than 5 Mb or that are closely spaced (24), although it has been proven as a robust and far-used method for the identification of cancer-associated copy number imbalances (25,26). SCNAs are assessed by hybridizing differentially labeled DNA from a tumor sample and reference genome (from normal individuals or match-paired patient's healthy tissue) to metaphase spreads. The ratio of tumor/reference competitive hybridization signals is used to determine the relative amount of tumor sequences with respect to normal counterpart along the chromosome segments.

The limit in resolution of these early techniques has been finally overcame only with the completion of the Human Genome Project (27) and the advent of DNA microarray-based technologies, such as aCGH and SNP-based arrays (28-31), leading to the explosion of information revealing the prevalence of SCNAs in cancer (32,33). Array CGH is based on the similar principle of traditional CGH, with the difference that a microarray chip of genomic probes has replaced the metaphase chromosome spreads as hybridization platform (28). In SNP array technology there is no comparative competition and sequence-specific oligonucleotides, homologous to the different SNP alleles existing in the human genome, are arrayed on a chip where fragmented test DNA is hybridized; depending on their SNP genotype, fragments bind only to the specifically-matched probe and give rise to a fluorescent signal detected by a scanner (30,31).

With a single-run experiment these array-based approaches can detect whole-genome SCNAs that would require hundreds of traditional tests, providing on top of that an empow-

ered resolution. The main limitation, however, of array-based techniques is the ability to detect aberration involving copy number changes only: balanced translocations and inversions are not detectable with this methodology. Furthermore, results of array experiments have to be generally validated, through FISH (23) or genomic real-time quantitative PCR, to confirm copy number changes of a specific test locus (34).

The sensitivity to detect genomic rearrangements has further escalated with the advent of NGS, or whole-genome sequencing (WGS), technology (35,36). This revolutionizing approach is potentially capable of providing information at single-nucleotide resolution of full cancer genomic mutation repertoire, including SCNAs, inversions, translocations, insertions, point mutations, breakpoints of SCNAs, and epigenetic changes in the DNA (35).

Finally, to correctly interpret the copy number data related to cancer genome, we must distinguish abnormal lesions from normal genomic variants present in the human population. Information about normal genomic variations are now publically available (Database of genomic variants, http://dgv.tcag.ca/dgv/app/home; Database of Single Nucleotide Polymorphisms, http://www.ncbi.nlm.nih.gov/SNP/) and, in principle, may be integrated with data of cancer genomic profiles, deposited in similar accessible database (UCSC Genome Browser, http://genome.ucsc.edu/; Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/; ArrayExpress, http://www.ebi.ac.uk/arrayexpress/; Integrative Genomics Viewer, http://www.broadinstitute.org/igv/) to crate a cohesive framework that will facilitate the understanding of cancer genomic complexity. Especially, The Cancer Genome Atlas (TCGA) demonstrates the power of integrative analyses coming from multiple experiments on a large series of cancer tissues (http://cancergenome.nih.gov/).

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION IN CANCER

During the past several years however, before the advent of NGS, aCGH has been extensively used as the gold-standard approach to assess genomic copy number changes in basically all human diseases (24,37,38). Especially, aCGH data have contributed enormously to the high-resolution mapping of specific genomic alterations and subsequent identification of target genes associated to cancer classification, disease progression, therapy response, and patient outcome in various tumor types (8,26,33). Particularly, oligo-based aCGH provided a wealth of information in cancer at genome-wide level with a cost-effective benefit compared to NGS and with

much higher resolution than previous methods (39,40).

The detection limit of aCGH depends on the probe density (the distance between each target distributed along the entire genome) and the size of the probes (41). This will establish the resolution of the chip used. The DNA probes range from genomic clones, most often bacterial artificial chromosome (BAC) clones (20-200 Kb), to oligonucleotides probes (20-80 bp). For example, if clones are spaced by 1 Mb intervals, everything that is smaller than 1 Mb will not be detected. With the introduction of oligonucleotide arrays the sensitivity has greatly enhanced: in a chip containing 1 million probes, potentially the sensitivity could be less than 500 bases, which is very close to highest-resolution SNP arrays (40,42).

In oligonucleotide aCGH analysis of cancer, total genomic DNA isolated from a tumor and normal control sample are labeled with two different fluorochromes (Cy5 and Cy3 respectively) and hybridized on a chip where the oligonucleotide probes corresponding to the human genome are randomly spotted (Fig. 1). The resulting ratio of the fluorescence intensity (usually expressed as log2 ratio) between the two fluorochromes at each DNA sequence is approximately proportional to the ratio of copy numbers of the corresponding genomic locus. Hybridization results are further analyzed by specific algorithms to distinguish differences between DNA copy numbers and the values are normalized so that the median log2 ratio is set to zero. Values of log2 around +1 indicate gain of one extra copy number (duplication) and above +2 indicate gain by a factor of four in copy number (amplification). Ratio near -1 indicates hemizygous deletion, while ratio below -2 (theoretically log2 of - ∞) indicates homozygous deletion (Fig. 1). Additional validation of the result is typically made by genomic quantitative PCR.

Although oligonucleotide aCGH arrays cannot identify breakpoints at the single-nucleotide level, recent high-resolution oligonucleotide platforms have narrow down considerably the accuracy of SCNA genotyping (39). An additional limit of aCGH technology is the impossibility to identify other types of genomic rearrangements than copy number changes, such as translocation and loss of heterozygosis (LOH). Nevertheless, for a number of different reasons, including friendly costs and manageable data analysis, aCGH remains the most convenient high-throughput method for precise genome-wide detection of SCNAs with direct diagnostic and clinical application (43).

Thanks to this technology, a number of regions that are frequently altered in cancer have been identified, giving a comprehensive picture of the most recurrent patterns of SCNAs (8). For example, there are common regions of gain/loss harboring well-established cancer-related genes that are the hallmark of many tumors. These may include gain and amplification of oncogenes such as *HRAS*, *CCND1* or deletion of tumor suppressors like *CDNK2A*, *PTEN* that can indeed be commonly observed across different tumor types (8,39). At the same time, characterization of tumor-specific genes, such as *NMYC* amplification in neuroblastoma (44,45), *ERBB2* amplification in mammary tumors (46,47), or *MITF*, the essential transcription factor involve in melanin production and melanocyte differentiation, in melanoma (48), have been exploited to draw a complete tumor classification based on SCNA profiles (49-52).

NEXT GENERATION SEQUENCING OF TUMOR GENOMES

The advent of NGS has brought a real revolution to the study of human genome biology (35). What initially had required over 13 years to sequence the entire human DNA now has incredibly reduced to potentially less than fifteen days (53,54). Most importantly, the rising of this technology has opened a new era of cancer genetics, starting in 2008 with the first cancer genome completely sequenced of a case of acute myeloid leukemia (55-57). Compared to previous method and array-based technology, NGS can provide a comprehensive picture of the cancer genome (58-60). In principle, single-nucleotide substitutions, small insertion or deletions, as well as chromosome rearrangements, including effects due to transposon elements, SCNAs, translocations, and chromosome insertions, can all be detected in a single run of reading (35,56). In addition, NGS is able to reveal mutations in non-coding regions, including promoters, enhancers, introns, non-coding RNAs, microRNAs (35,58), and epigenomic changes (61).

The experimental approach of NGS is based not on a unique technique but consists of a broad number of different methods required for the template preparation, imaging of the signals and data analysis (59,62). The combination of specific protocols distinguishes the type of data retrievable from the different platforms available (62,63). Major differences are in the template preparation however; although a common theme is that the template DNA is amplified to form a library, which is then immobilized so to allowed thousands of sequencing reaction to happen simultaneously (63). Individual DNA fragments of the library, originated from a single molecule of genomic DNA, are amplified either on beads or on flat surface prior to sequencing reaction and each
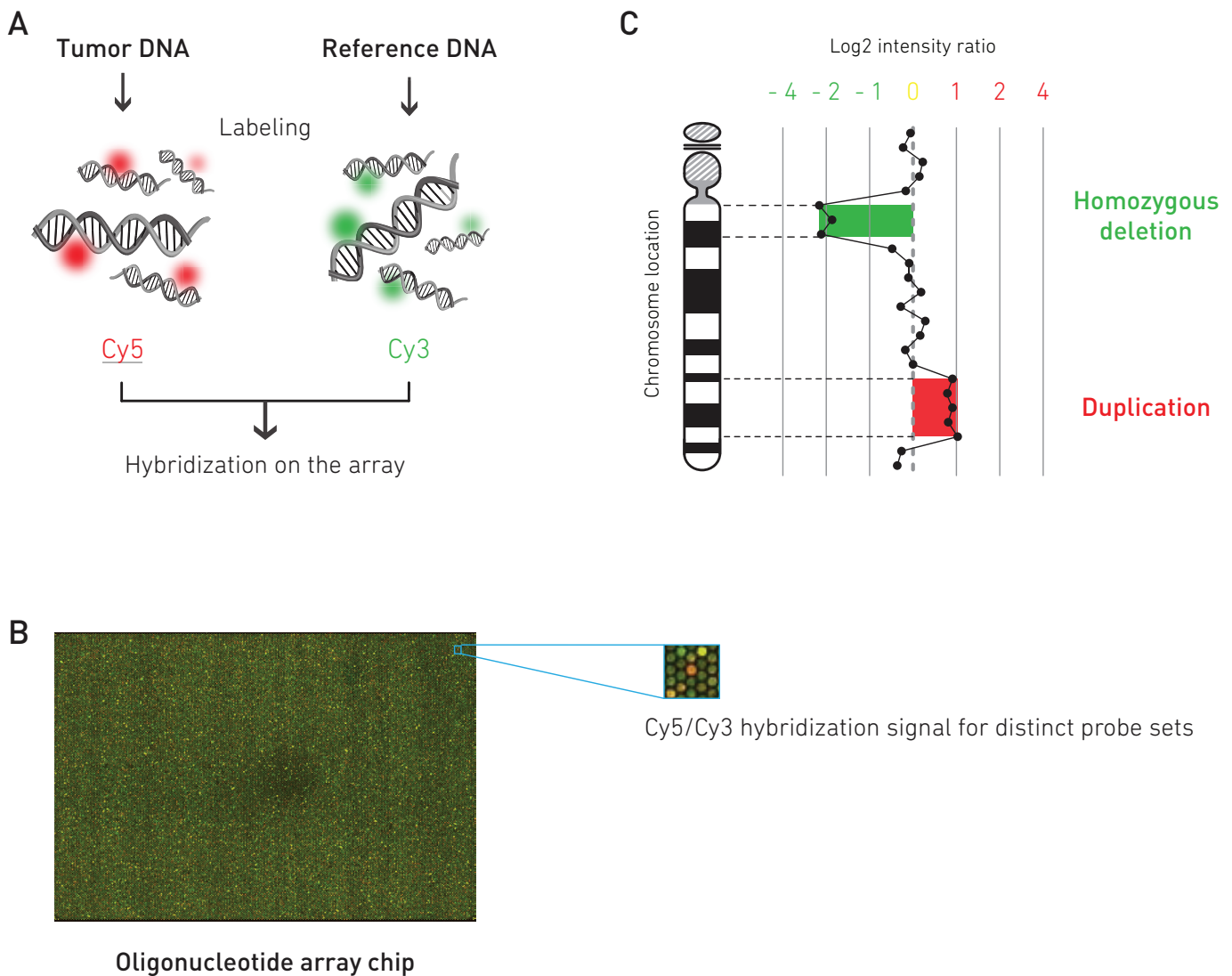
Figure 1. *Schematic representation of the procedure used to assess SCNAs in tumors by oligonucleotide aCGH. **A.** Array CGH involves competitive hybridization of tumor test DNA and reference diploid DNA samples (differentially labeled with red Cy5 and green Cy3) to a pre-designed set of probes covering the entire genome, which are spotted on a chip microarray slice. **B.** For each gene or genomic sequence included in the array, the hybridization signal is a result of the average intensity from multiple probes representing the same gene, spotted in a specific well on the array. The image shows an aCGH experiment of a MM patient (labeled red) compared with normal tissue (labeled green). Red color indicates increased DNA copy number, green represents loss of genomic sequence (that is a deletion), while yellow represents no changes (diploidy). Inset, red/green hybridization signals are represented for selected spots. Unpublished data, courtesy of A. Quattrone (Laboratory of Translational Genomics, CIBIO, University of Trento, Italy). **C.** The Cy5/Cy3 ratios of signal intensity are then measured for each probe (generally as log2 ratio) and normalized so that significant deviations from zero indicate variation (loss or gain) in the copy number of that particular DNA fragment in the test sample, relative to the reference, as exemplified here.*

MM, malignant melanoma; SCNA, somatic copy number alteration; aCGH, array comparative hybridization.

sequence read is equivalent to a single DNA fragment (63). For a detailed explanation of these technical procedures and descriptions of commercially available platforms, I would refer the reader to specific reviews (35,56,62,63).

Over the past two years, NGS technique has given a great boost to the field of cancer genome. In the investigation of SCNAs, NGS offer substantial benefits over aCGH, including higher resolution (up to the level of single-nucleotide insertion/deletion while the minimum current resolution of array methods is the order of 0.1-0.5 kb) and precise localization of the breakpoints that cause SCNAs (59,63). Moreover, unlike aCGH, measurements taken by NGS do not suffer of intensity saturation problems and therefore permit a more accurate estimation of the signals (62). Another important advantage of NGS lays in the possibility to detect rearrangements in highly repetitive sequences (Alu, LINE, centromeres) that were previously totally missed by aCGH analysis (56). Finally, before the NGS technology, there was no high-throughput method to define precise sites of chromosomal rearrangements, like inversions and translocations, which were roughly identified by cytogenetics only in specific subset of tumors where those are particularly frequents, such as chronic myeloid leukemia and sarcoma (64).

One major potential of genome sequencing for modern cancer research is possibly the rapid discovery of novel and chromosomal rearrangements. However, major applications of NGS are oriented to targeted sequencing of a region of interest, like exon sequencing, which can be executed at lower costs and higher throughput way compared to a whole-genome shotgun sequencing. In cancer, transcriptome sequencing (RNA-seq) is a selective and efficient approach to detect intragenic fusions, point mutations, and changes in gene expression in a single-run experiment, especially for transcripts with low-level expression (60,64-66). Additionally, NGS has moved further ahead from just DNA sequence analysis toward a number of different applications that have considerably improved our understanding of nucleic-acid biology and gene expression mechanisms (67).

Altogether, with the aim to define new approaches for the discovery of variants associated with specific response to therapy (68) and predisposition to disease (69), NGS technology has found an important application in the genome-wide association studies (GWAS) (35). Comparing allele frequency across the genomes of a large number of cases, thousand of genomic variants linked to the clinical heterogeneity in cancer can be found (58,70), opening the road to individualized tumor treatment (69,71).

Overall, DNA cancer sequencing data have been collected in public-assessable repositories, among all TCGA database (72). Also, a complete catalogue of cancer-associated alterations can be found at the COSMIC database, which stores a vast amount of information directly related to the publication links (www.cancer.sanger.ac.uk/cancergenome/projects/cosmic).

COPY NUMBER ALTERATION ANALYSIS IN MALIGNANT MELANOMA

The following paragraph of this review is focus to the advances obtained in the study of SCNAs in cutaneous malignant melanoma (MM). The majority of studies have been based on conventional CGH and aCGH (73,74), but recent introduction of NGS and the integration with previous mRNA expression and point-mutation data (75,76), may expand incredibly the information, concretizing the possibility to define predictive tools of patient outcome and therapy resistance (77,78).

Cutaneous MM is the most common cause of death (about 65% of deaths) related to skin cancers and, despite accounting for some 4% of skin cancers and average 2% of all tumors, its incidence is dramatically increasing (Fig. 2A); more than 60 000 new cases a year are diagnosed in Europe with over 10 000 estimated death (Fig. 2B), and the rate in North America and Australia is even higher (79,80). Primary tumor biopsy followed by histological inspection have long been considered the gold standard for diagnostic guidance (81,82) of MM, and thickness (Breslow thickness), denoted as the depth of tumor invasion, is a major independent indicator of neoplastic progression (83). In addition, since sentinel lymph nodes (SLNs) are the most likely primary site of metastasization, sentinel-node biopsy is routinely used as the minimal essential procedure to evaluate the clinical prognosis (84). When detected at its early stages, cutaneous MM is almost 90% curable (83). However, due to the total lack of symptoms during the initial phase of invasion and the difficulty to discriminate ambiguous lesions using conventional histologically criteria, cutaneous MM is often recognized after metastasization to distant organs, when the prognosis is definitively poor (83) and treatments are usually restricted to lymphadenectomy and postoperative non-curative chemotherapy (85,86). In this context, the introduction of molecular tests for SCNAs analysis has brought the diagnosis of the disease a step higher (73,77) and, with the advantage of genomic pro-
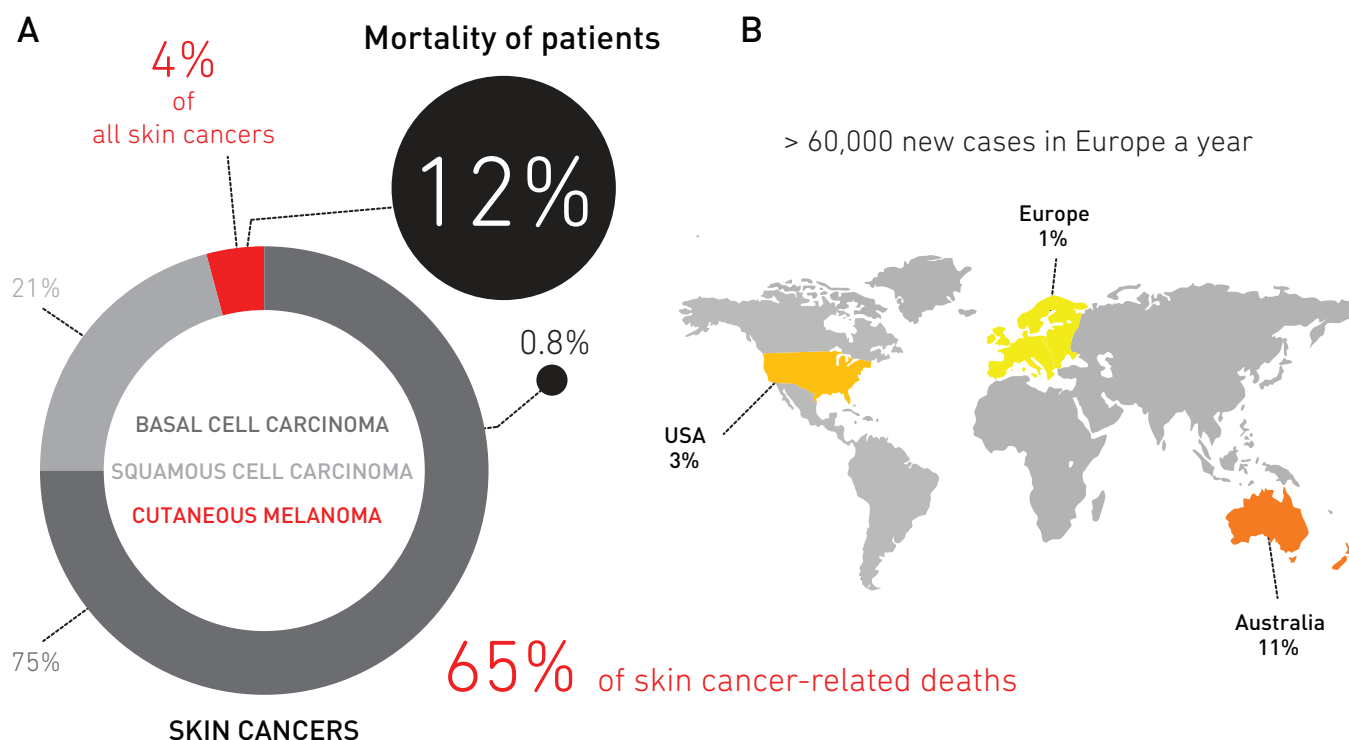
Figure 2. *Incidence and mortality of cutaneous MM. **A.** Incidence of cutaneous MM over other skin cancers. Basal cell carcinoma is by far the most frequent form of cancer in the human population worldwide and accounts for the 75% of tumors developing on the skin. However, basal cell carcinoma rate of date (0.8%) is more than ten-folder lower than the mortality rate associated to MM, where some 12% of patients affected by the disease die. MM represents only the 4% of all skin cancer but causes 65% of skin cancer-related death (80). **B.** Cutaneous MM incidence as percentage of total cancer cases in westernized countries. Recurrence of cutaneous MM is directly linked to sun exposure and light-skin phenotype, which explains its elevated frequency in Australia and New Zealand, where MM represents the second most common tyoe of cancer. In Europe, 60,000 total new cases are diagnosed every year (79,80).*

MM, malignant melanoma.

filing, it has become clear the existence of genomic instability patterns of highly recurrent SCNAs typically associated to this cancer (87).

Malignant melanoma is a tumor that already at its primary condition shows profound patterns of SCNAs as revealed by aCGH (Fig. 3) and the association of specific SNCAs to known histopathological parameters and tumor progression phenotypes has long been pursued to the scope of defining early-development molecular biomarkers (52). Among all, *CDKN2A* is the first gene discovered that associates to fa-

milial susceptibility of developing MM (88,89) and the most common somatically deleted gene in MM (87,90,91). The importance of p16-RB pathway in MM is reinforced by the elevated frequency of *CDK4* amplification (90,91). Other frequent SCNAs in MM are losses of regions in 6q23, 8p, and 10q and gains involving 1q, 4q12, 5p15, 6p25, 7q, 8q, 11q13, 12q14, 17q, 19p, 20q and 22q (87,90-93). For most of these regions, the driver genes have not been identified, though other recurrent melanoma SCNA-driving genes have been characterized (87), including *CDKN2A* (deletion
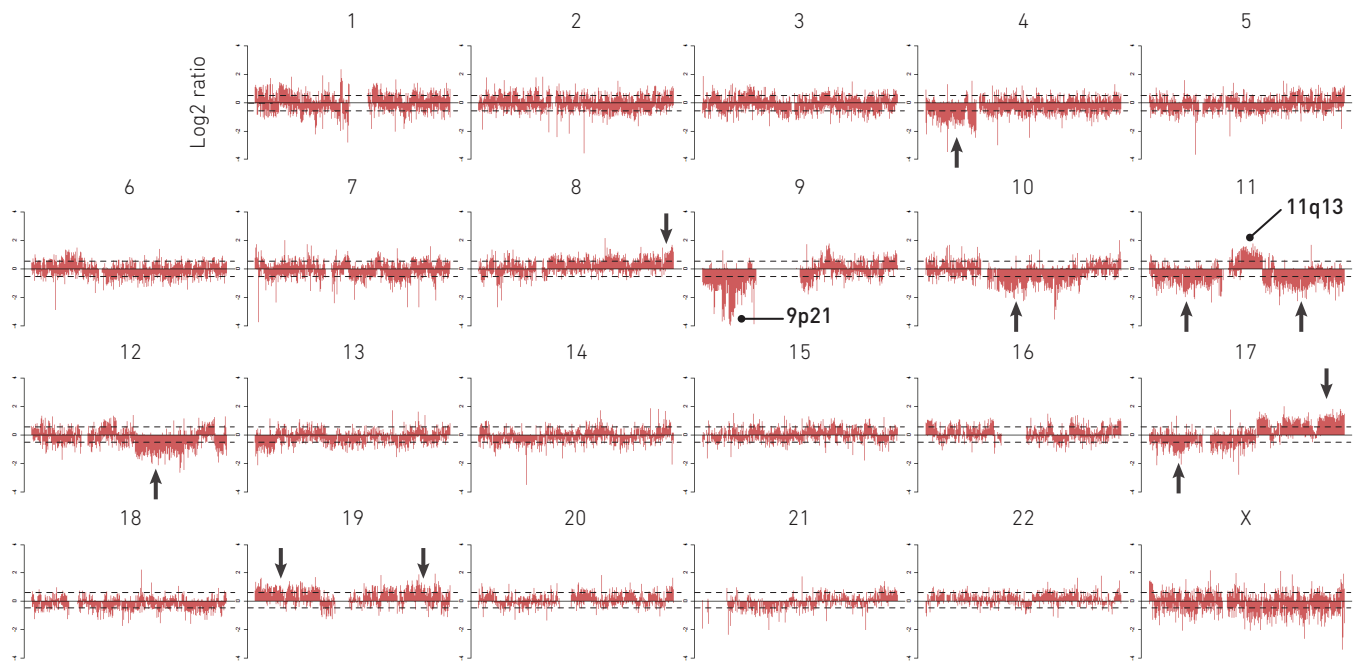
Figure 3. An example of genomic aCGH profile of a primary MM associated with worst prognosis obtained by using Agilent oligonucleotide array (Agilent Technologies, Santa Clara, CA). Most of the chromosomes reveal profound pattern of SNCAs. For each chromosome, the intensity of raw hybridization signals, between tumor DNA and diploid sex-matched control, is reported as log2 ratio (ordinate) plotted against chromosome map position (abscissa). Chromosome Y is not included in the analysis. Dashed lines indicate the upper and lover thresholds of diploid copy number. Gross genomic gains and losses are evident over the threshold bars (pointed by the arrows). Clear deletion of the entire 9p arm is present, including homozygous deletion (log2 ratio < -2) of 9p21 where CDKN2A is located. DNA duplication is detected at the locus of CCND1 (11q13). Unpublished data, courtesy of A. Quattrone (Laboratory of Translational Genomics, CIBIO, University of Trento, Italy).

MM, malignant melanoma; SCNA, somatic copy number alteration; aCGH, array comparative hybridization.

of 9p21), *PTEN* (deletion of 10q23), *KIT* (amplification of 4q12), *CCDN1* (amplification of 11q13), and *CDK4* (amplification of 12q14). Often, type and number of SCNAs correlate with the specific histological subtypes (90,92,94). Furthermore, in this specific type of tumor, SCNAs analysis allowed a detailed sub-classification according to skin location of the primary site and exposure to UV, thus improving the disease diagnosis and bringing to the identification of subsets of patients with different clinical coursers (73,90). Comparison of tumor samples at different stages, such us premalignant or *in situ* lesion, invasive cancer, and metastatic disease, has also demonstrated that the overall number of SCNAs increases during tumor progression (76,95). Figure 4 shows some examples of SCNAs that are commonly associated to primary MMs as revealed by oligonucleotide aCGH.

Among all alteration associated to MM, constitutive activation thought RAS/MAPK signaling is the leading cause of tumor progression, although the main genes involved in this pathway are preferentially mutated than amplified or gnomically altered (96). Specifically, MM displays the highest rate of constitutive kinase-activating mutations in *BRAF* gene over all human tumors (97,98) and *NRAS* is mutated in almost 15% of cases (98). At the same time, activation of PI3K/AKT cascade occurs in almost 30-40% of cases (96). The main event that causes deregulation of PI3K pathway is the deletion of *PTEN* (99), which is usually the second most recurrent homozygously deleted gene after *CDKN2A* in MM (90,100). Possibly, loss of *PTEN* is considered an essential step in the oncogenic progression of BRAF-mutated melanocytes (101). This has made, AKT and MAPK pathways, and
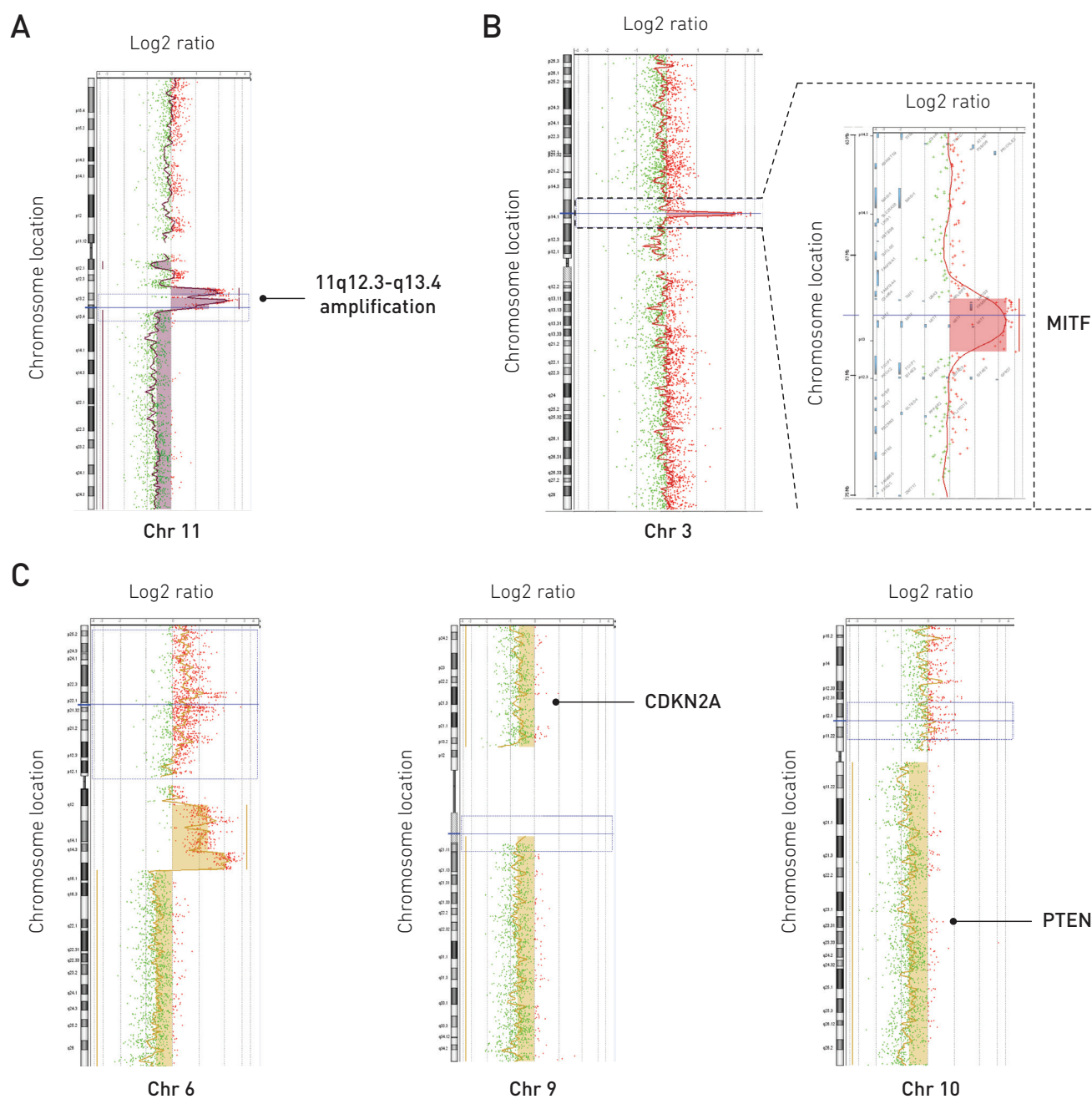
Figure 4. *Visualization of genomic aCGH result for single chromosomes of primary MM samples. Data were obtained by using Agilent oligonucleotide array and filtered with Agilent Genomic Workbench software (Agilent Technologies, Santa Clara, CA). Intensity of the hybridization signals is reported as log2 ratio between tumor DNA and sex-matched diploid control (abscissa) and plotted against the chromosome map position (ordinate). Dashed lines indicate the upper and lover thresholds of gain/loss copy number. Color-filled areas denote a gain (right) of a loss (left).* **A.** *A patient showing amplification of region 11q13, where CCND1 gene is located, and concomitant deletion of adjacent segments.* **B.** *A patient showing focal amplification of MITF gene on chromosome 3.* **C.** *A patient with complete deletion of chromosome 9 and chromosome arm 10q, potentially driven by loss of CDKN2A and PTEN gene respectively, and a distinct breakpoint on chromosome arm 6q (arrow) that has generated an amplification followed by loss of nearby material. Unpublished data, courtesy of A. Quattrone (Laboratory of Translational Genomics, CIBIO, University of Trento, Italy).*

MM, malignant melanoma; aCGH, array comparative hybridization.

BRAF in particular, the most pursued clinical targets for the development of new drugs (102). A good fraction of patients with BRAF mutation seems to respond positively to specific targeted inhibition (103). However, it has been recently discovered that tumor growth can be enhanced in the presence of RAS mutation by using BRAF inhibitors when BRAF is actually not mutated (104). Acquired resistance to BRAF-targeted therapy may also depend on specific SCNA background and selective amplification of mutated-BRAF itself (105). Interestingly, mutation of *CDK4* gene confers resistance to BRAF inhibitors and this apparently only happens in concomitance with amplification of *CCND1* (106). These finding further highlight the importance of accurate SCNA genome typing of individual patients in the clinical practice before making decision of therapeutic strategy.

Recent NGS whole-genome sequencing analyses have re-inforced the accuracy of genomic alteration profiling of MM confirming most of the know SCNAs and posing the basis for the identification of novel MM-related genes (60,76,107,108). These studies have already provided a weight of information for the identification of a number of driving genes directly attributable to UV exposure for some of the most common SCNAs (60,76,107). Comparison of primary tumor and related metastasis has then proved the elevated signal accuracy of NGS with respect to array analysis of the same samples and revealed novel specific SCNAs that are acquired only by the metastatic tumor (108).

Finally, a global approach of integrative analysis of melanoma genome sequencing and associated RNA-sequencing data has brought to the discovery of novel potential targetable molecules for therapeutic strategies (109). The integration of transcriptome sequencing data with accurate SCNAs profiling and the association to the presence of important driving mutations, such as those in *CDK4*, *BRAF* or *RAS* genes, is at the moment only at the research level (76) but in the future might represent the scaffold for the development of appropriate therapies.

CONCLUSION

Structural polymorphism of DNA may have played a fundamental role in human evolution, by determining genetic diversity within the population, and have a significant part in disease development and predisposition. Germline CNVs across healthy individuals are widely distributed throughout the genome. However, some hotspot events are recurrent in regions of known somatic rearrangements under strong adaptive selection, like during tumorigenic progression. Profiling of driving cancer genetic alterations at increasingly higher resolution has been pursued intensively over the last decade bringing to the notion that SCNAs are a hallmark of cancer. Comprehensive high-resolution view of SNCAs in various tumor types, acquired with the introduction of NGS, has offered a better understanding of cancer development and provides improved tools for the definition of novel diagnostic signature and therapeutic targets.

Identification of specific SCNAs for each patient that can be predictive of prognosis and guide pre-evaluation of therapeutic response is the goal in the clinical management of cancer. Particularly in those tumors, like cutaneous MM, that can be easily detected by dermatological inspection and rapidly removed from the primary site on the skin. High-throughput characterization of whole-genome SCNA profile of individual primary melanomas may become a real possibility in the next future and can provide immediate prognostic information to decide the appropriate therapeutic options to be selected for clinical trials.

It is much probable that the ongoing innovation of genome sequencing will likely continue to advance and will continue to transform our view of cancer alteration landscape. Also, the increased availability of public data through The Cancer Genome Atlas will make available for the analysis by independent groups an incredibly huge cohort of clinical samples. Translational genomics based on the integration of such a great amount of shared information will open the route to precision medicine of a disease that is extremely heterogeneous at the individual level. I would envisage a personalized sequencing approach for cancer diagnosis, based on the comparison of inherited and somatically acquired genetic changes, to establish the best-tailored treatment for each patient.

ACKNOWLEDGMENTS

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